100%O_2 (Normo) or 100%N_2 (Hypo). We recorded XRD patterns and LV pressure (LVP) at end-diastolic LVP (EDP) of 0 and 20 mmHg. **Results**: Under Normo, increasing EDP significantly increased the developed LVP (EDP0: 104 ± 26mmHg vs. EDP20: 141 ± 25mmHg, $p<0.01$). Under Hypo, developed LVP and its duration did not show significant differences compared with those in Normo. However, increasing EDP under Hypo significantly decreased developed LVP (72 ± 13mmHg vs. 96 ± 16mmHg, $p<0.01$). The minimum value of the (1,0)/(1,1) intensity ratio (I_{min}) provided by the XRD analysis was used as an index of AMI. I_{min} showed a significantly negative correlation with developed LVP regardless of Normo or Hypo. The diastolic myosin filament lattice spacing (MFL) calculated from the diffraction angle of the (1,0) equatorial reflection would be reduced by increasing EDP. In contrast to a significantly positive MFL-I_{min} correlation under Normo, we observed the significantly negative MFL-I_{min} correlation under Hypo. We confirmed that the duration of Ca^{2+} transient was slightly longer but the amplitude of Ca^{2+} transient was unchanged under Hypo. **Conclusion**: These novel findings suggest that under Hypo the probability of AMI decreases even though the MFL was reduced with increasing preload. This is an underlying mechanism for reduced cardiac contractile performance under hypoxia.

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Influence Of Acidic pH On The Rate Of Force Development In Cardiac Muscle

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Cellular acidosis, a consequence of myocardial ischemia, reduces the Ca^{2+} sensitivity of cardiac contraction and maximal Ca^{2+} activated force in cardiac muscle. These effects are similar to that seen in fast (psoas) and slow (soleus) skeletal muscle with reduced pH. Previous studies have also demonstrated no effect of low pH on the rate of force redevelopment (k_{tr}) at maximal Ca^{2+} activation in slow and fast muscle fibers, but that k_{tr} is slowed at submaximal Ca^{2+} activation. However, it is unknown whether low pH affects calcium dependence of k_{tr} in cardiac muscle. To characterize the influence of acidic pH on k_{tr} we have measured Ca^{2+} activation of skinned cardiac trabeculae at pH 7.0 and 6.5. As in skeletal muscle, reduced pH significantly decreased isometric force in cardiac muscle at all levels of Ca^{2+} activation ($\Delta pCa_{50} = 0.73$). Interestingly, in contrast to skeletal muscle, k_{tr} at low pH in cardiac trabeculae was significantly faster at both maximal (pH 7 = 5.1 ± 0.5 s^{-1}, pH 6.5 = 6.9 ± 0.3 s^{-1}) and half-maximal (pH 7 = 3.0 ± 0.3 s^{-1}, pH 6.5 = 6.6 ± 0.2 s^{-1}) Ca^{2+} activation. This is consistent with previous studies showing increased force redevelopment in cardiac muscle when force is inhibited with phosphate, vanadate, or reduced sarcomere length. Our results support the idea that k_{tr} is negatively correlated to the size of the cross-bridge pool available for recruitment to cooperative activation of the thin filament. Force inhibition such as that seen with lower pH may reduce the cross-bridges available for recruitment, which would reduce this slowing effect and speed force redevelopment.. Supported by NIH R01 HL 65497 (MR), T32 HL07828 (FSK) and NSERC Discovery (TEG).

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Role of Strongly-Bound Crossbridges in Cooperative Cardiac Thin Filament Activation

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Experimental evidence indicates that activation of cardiac thin filaments is enhanced by strongly-bound myosin crossbridges and that crossbridge binding is in turn cooperatively regulated by end-to-end interactions between adjacent tropomyosins. We examined the impact of crossbridge binding and nearest-neighbor tropomyosin interactions on thin filament activation using a computational model.

We represented individual thin filament regulatory units (RUs) with the model of McKillop and Geeves [Biophys J, 1993 65(2)] wherein RUs are found in blocked (B, non-permissive), closed (C, permissive), and open (M, permissive with crossbridge) states. The B to C transition was assumed to depend upon Ca2+ concentration. Nearest-neighbor RU interactions were represented by causing transitions of the individual RU model to depend on the status of neighbors. Ensembles of N interacting RUs were modeled as Markov networks generated by considering all possible unique configurations of individual RUs (B, C, or M) within a chain.

The model-generated steady-state force-pCa curve (N=6) possessed a Hill coefficient of 3.0. Hill coefficients fit separately to portions of the curve below and above half activation were 3.2 and 2.4, respectively. Rate of force redevelopment following rapid slack/restretch (ktr) showed strong dependence on activation level (ktr=2.6 s-1 at pCa 6.0 vs. 9.3 s-1 at pCa 4.3). Increasing the cross-bridge duty cycle in the model increased myofilament Ca2+ sensitivity but had an opposite effect on Ca2+ sensitivity of ktr. Simultaneous matching of reported force and ktr sensitivities required a duty cycle of 30%. Increasing N toward 26 (a realistic filament length) tended to improve the fit with experiments. These results suggest that cycling crossbridges act through nearest-neighbor interactions along the thin filament to 1) increase myofilament Ca2+ sensitivity, 2) cooperatively enhance activation, and 3) slow the rate of force redevelopment at low levels of activation.

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Kinetics of ADP Release From Cycling Cross Bridges In Contracting Skinned Cardiac Muscle Monitored With A Fluorescent Probe

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Kinetics of ADP release from cycling cross bridges were studied in Ca activated skinned papillary muscle by displacement of fluorescent ADP bound to the cross bridges in AM*ADP state(s) by non-fluorescent ADP photogenerated from caged ADP. A strip of glycerinated papillary muscle (100um, 2mm) from guinea pig left ventricular in Ca-free solution was loaded with a mixture of non-fluorescent ATP (1mM), fluorescent 3′-amino-deoxy ATP (aminoATP) (50μM) and 5 mM caged ADP in the presence of an ATP back-up system. At the plateau of force at pCa5.8 the muscle was rapidly transferred into the photolysis trough filled with silicone oil and irradiated by a 437 nm laser pulse. Alternatively, a muscle loaded with only fluorescent amino ATP (50μM) was allowed to contract at pCa4.5 in oil and develop rigor with amino ADP bound to the cross bridges. Following photolysis of caged ADP the kinetics of force and fluorescent transients were found markedly different in contracting and rigor muscles. In contracting muscle the force and fluorescence both increased following caged ADP photolysis, while in rigor muscle the photolysis induced an increase in fluorescence, but decrease in force. Kinetics of ADP release estimated by the rate of fluorescence increase was significantly slower in contracting muscle than that in rigor: 2-4 s-1 vs 18-20s-1, suggesting that at least two different AM*ADP states exist during ATP hydrolysis by cycling cross bridge in contracting papillary muscle. Supported by NIH grant R03 AR05 2885 for A.K.

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Mechanoenergetics of Actomyosin Interaction Analyzed by Cross-Bridge Model

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We present a mathematical model of actomyosin interaction, as a further development of actomyosin model that links mechanical contraction with energetics (Vendelin et al, Annals of Biomedical Engineering: 28, 2000). The new model is a three-state Huxley-type model, with two strong binding states and one weak binding state, for cross-bridge interaction and a model of calcium induced activation. The force produced by the attached cross-bridge in strong binding state is assumed to be elastic and depends linearly on the axial distance z along the myosin and actin filaments between the equilibrium position of the myosin head and the nearest actin binding site. The model is self-consistent and is based on T. Hill formalism linking free energy profile of reactions and mechanical force.

In several experimental studies it has been shown that the dependency between oxygen consumption and stress-strain area is linear. Additionally, the relation between stress-strain area and oxygen consumption is the same for isometric and shortening contractions. In this work, we analyzed free energy profiles of actomyosin interaction by changing free energies of intermediate states and free energies of activation of different reactions.

In our simulations we replicated the linear dependence between oxygen consumption and stress-strain area together with other important mechanical properties of cardiac muscle such as developed stress dependence on the sarcomere length and force-velocity relationship.

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Increasing Heart Rate Decreased Actin-Myosin Interaction in Isolated Beating Rat Whole Heart

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Background: Heart rate (HR) is one of the determinant factors of cardiac performance. Failing human myocardium shows negative force frequency relation, whereas normal one shows positive relation. **Purpose**: To test the effect of HR on actin-myosin interaction (AMI) in beating rat hearts those have negative